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Yersinia specific immune complexes in the synovial fluid of patients with yersinia triggered reactive arthritis

RIITTA LAHESMAA-RANTALA,[1] KAISA GRANFORS,[1] HEIKKI ISOMÄKI,[3] AND AULI TOIVANEN[1 2]

From the Departments of [1]Medical Microbiology and [2]Medicine, Turku University, Turku; and the [3]Rheumatism Foundation Hospital, Heinola, Finland

SUMMARY Yersinia specific immune complexes were demonstrated in the synovial fluid of three patients out of 12 with yersinia triggered reactive arthritis. They were not detectable in the synovial fluid of any of the 16 control patients, including nine with reactive arthritis triggered by factors other than yersiniae. Platelet reactive IgG was detectable in the synovial fluid of eight out of the 12 patients with yersinia triggered reactive arthritis and in three of the 16 control patients, all three having rheumatoid arthritis. An enzyme linked immunosorbent assay and a platelet ^{125}I labelled staphylococcal protein A test were used to measure yersinia specific immune complexes and platelet reactive IgG respectively. The results obtained show for the first time the occurrence of bacterial antigens, derived from the causative strain, in the synovial fluid in yersinia triggered reactive arthritis.

The pathogenesis of yersinia triggered reactive arthritis remains unknown. HLA-B27 is known to be associated with the development of this postinfectious complication, and certain interesting features characterise the immune response of the patients.[1 2] For instance, circulating immune complexes (ICs) containing IgM and yersinia antigens occur more often in higher concentrations in patients with arthritis than in those without postinfectious complications.[3] In some of the patients the ICs persist even up to one year after onset of the infection. The role of the ICs in the development of reactive arthritis remains unknown, however.

One of the main criteria for the pathogenetic significance of circulating ICs is their occurrence in the target organs.[4 5] For yersinia triggered reactive arthritis an investigation has been reported in only two cases,[6] in both of which synovial fluid ICs were detected by the platelet aggregation test; the nature of the antigen involved was not studied. In the present work we have studied the occurrence of yersinia specific and non-specific ICs in the synovial fluid of patients with yersinia arthritis.

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Correspondence to Dr Riitta Lahesmaa-Rantala, Department of Medical Microbiology, Turku University, SF-20520 Turku, Finland.

Patients and methods

PATIENTS AND SAMPLES

Synovial fluid was obtained from 12 patients, 10 men and two women, with reactive arthritis triggered by *Yersinia enterocolitica* O:3 (Table 1). Yersiniosis was diagnosed on the basis of clinical symptoms, serology, and bacterial cultures.[3 7] All patients developed typical reactive arthritis after the initial infection, with one or more joints involved. All the synovial fluid samples were taken from the knee two to 11 weeks after onset of the initial infection. Synovial fluid cultures of blood and chocolate agar were negative for micro-organisms from all samples. For controls, fluid was taken from 16 patients with knee joints swollen for other reasons; nine had reactive arthritis triggered by factors other than yersiniae (Table 2). All synovial fluids were centrifuged within one hour to remove cells and fibrous clots, and the supernatants were stored in small aliquots at −40°C until used.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR YERSINIA SPECIFIC ICs

A recently developed ELISA for the detection of yersinia specific ICs according to the immunoglobulin class involved was applied.[8] In the assay yersinia

Table 1 *Clinical features of the patients with yersinia triggered reactive arthritis*

Patient No	*Age (years)*	*Sex*	*HLA-B27*	*Stool culture*	*Clinical symptoms*			*ESR† (mm/h)*
					Diarrhoea	*Arthritis*		
						Acute	*Chronic**	
1	46	M	+	−	Yes	Yes	No	77
2	44	M	+	−	Yes	Yes	No	120
3	30	M	+	O:3‡	Yes	Yes	No	72
4	41	M	+	−	Yes	Yes	No	85
5	45	M	+	−	Yes	Yes	No	94
6	17	M	+	O:3	No	Yes	No	55
7	16	F	+	−	Yes	Yes	Yes	37
8	27	M	+	−	Yes	Yes	No	100
9	31	M	+	O:3	Yes	Yes	No	80
10	27	M	+	O:3	No§	Yes	Yes	75
11	38	F	+	−	Yes	Yes	No	97
12	39	M	+	O:3	Yes	Yes	Yes	66

All patients showed strong antibody response against *Yersinia enterocolitica* O:3 (IgM, IgG, and/or IgA as measured by enzyme immunoassay).
*Symptoms persisting for more than a year after the initial infection.
†The value observed at the time when synovial fluid was taken; ESR=erythrocyte sedimentation rate.
‡*Yersinia enterocolitica* O:3.
§Only abdominal pain.

Table 2 *Clinical features of the control patients*

Patient No	*Age (years)*	*Sex*	*HLA-B27*	*Diagnosis*	*Trigger of ReA**	*ESR (mm/h)*
1	53	F	ND	ReA*	Salmonella*	33
2	41	M	ND	ReA	Salmonella	36
3	28	M	+	ReA	Campylobacter*	71
4	34	F	+	ReA	Streptococcus*	53
5	41	F	−	ReA	Ulcerative colitis	53
6	46	M	+	ReA	Unknown	100
7	21	M	ND	ReA	Unknown	71
8	22	F	+	ReA	Unknown	75
9	21	M	+	ReA	Unknown	59
10	27	F	ND	RA*		48
11	55	M	ND	RA		31
12	69	M	ND	RA		26
13	20	M	ND	Juvenile RA		19
14	65	F	ND	Arthrosis of the knee		18
15	35	M	ND	Distension of the knee		24
16	41	M	−	Hyperuricaemia		36

*ReA=reactive arthritis; Salmonella=*Salmonella typhimurium*; Campylobacter=*Campylobacter jejuni*; Streptococcus=β haemolytic streptococcus, group A; RA=rheumatoid arthritis.

ICs are captured by the antibody to polystyrene microtitre plates by rabbit antihuman immunoglobulins, and the existence of *Y entercolitica* O:3 antigens is demonstrated using Fab fragments of alkaline phosphatase conjugated antibody against the same serotype. Samples were diluted 1:20 in phosphate buffered saline (0·1 M, pH 7·5) containing 5% normal rabbit serum. As control each plate included a positive serum with a high concentration of yersinia-immunoglobulin complexes of all three immunoglobulin classes, buffer, and a pool of sera from 50 healthy blood donors. Each sample from the healthy controls was also analysed separately to calculate a mean and standard deviation (SD). Optical density values at 405 nn (OD_{405}) exceeding by 2SD the values for the pooled normal controls present in each assay were taken as positive. All assays were performed in duplicate.

PLATELET ^{125}I LABELLED STAPHYLOCOCCAL PROTEIN A TEST (PIPA)

Platelet reactive IgG, usually considered to repre-

sent antigen non-specific ICs, was determined by a platelet ^{125}I labelled staphylococcal protein A test (PIPA). The details of the test are described elsewhere.[9] Human platelets were allowed to react with 1:10 diluted synovial fluids on microtitre plates. After washing, the binding of IgG through the Fc receptors to the test platelets was measured by radiolabelled protein A. Results were expressed as SD units, calculated from a panel of normal sera included in each test. Values of more than 2SD above the mean for normal sera were considered positive.

DETERMINATION OF RHEUMATOID FACTOR

Rheumatoid factor was determined in all samples from patients with yersinia triggered reactive arthritis by an ELISA for IgM rheumatoid factor.[10]

Results

In the patients with yersinia triggered reactive arthritis yersinia specific ICs were demonstrated in three out of 12 synovial fluids (Table 3). One patient (No 10) had a high level of yersinia IgM ICs (OD_{405} 0·778) and another patient (No 8) a low level (OD_{405} 0·406). Yersinia IgA ICs were observed in one patient (No 7) at a low level (OD_{405} 0·391). Assay for yersinia-IgG ICs was negative in all samples. All synovial fluid samples taken from the 16 control patients were negative for the yersinia specific ICs of all immunoglobulin classes.

Table 3 *Occurrence of immune complexes (ICs) in the synovial fluid of patients with yersinia triggered reactive arthritis*

Patient No	*Yersinia specific ICs**			*Platelet reactive IgG†*
	IgM	*IgG*	*IgA*	
1	0·260	0·190	0·226	<2·0
2	0·288	0·278	0·252	3·0
3	0·234	0·234	0·226	19·4
4	0·296	0·274	0·250	<2·0
5	0·189	0·270	0·212	21·8
6	0·275	0·289	0·298	<2·0
7	0·295	0·262	0·391	6·8
8	0·406	0·292	0·255	<2·0
9	0·277	0·269	0·258	4·0
10	0·778	0·268	0·230	16·0
11	0·234	0·196	0·239	9·4
12	0·209	0·258	0·206	12·4

*Yersinia specific ICs of different immunoglobulin class were measured by an ELISA. The results are expressed as optical density values at 405 nm. The limit for positivity (2SD over the mean of 50 normal controls) was 0·356 for yersinia-IgM ICs, 0·535 for yersinia-IgG ICs, and 0·385 for yersinia-IgA ICs. The values exceeding the limit are underlined.
†Platelet reactive IgG measured by PIPA is expressed as SD units. Values exceeding 2SD were considered positive (underlined).

A PIPA was positive in eight out of the 12 synovial fluids taken from patients with yersinia triggered reactive arthritis (Table 3). The synovial fluid of the three control patients with rheumatoid arthritis (Nos 10–12) had positive PIPA values of 7, 16, and 10 SD units respectively. In all other control samples PIPA values remained negative.

Determinations of IgM rheumatoid factor in synovial fluids taken from patients with yersinia triggered reactive arthritis were negative.

Discussion

Our findings indicate the existence of yersinia antigens in the form of ICs in the synovial fluid of patients with reactive arthritis after yersiniosis. We also believe that the occurrence of ICs containing yersinia antigens in the synovial fluid in reactive arthritis is more common than shown by the present study (in three out of 12 patients) since different methods detect different ICs.[11] This holds true for the ELISA of yersinia specific ICs and for the PIPA. PIPA detects platelet reactive IgG, which consists in the serum of antigen-IgG complexes, IgG aggregates, and sometimes also specific antibodies, e.g., antiplatelet and anti-HLA antibodies.[12] For the ELISA of yersinia specific ICs that we used the antigen within the ICs must be exposed to be detected.[8] Also, only one rabbit anti-yersinia serum was applied; use of antisera detecting other epitopes might have increased the number of positive samples. Therefore, synovial fluids positive only with a PIPA may contain yersinia antigens as well. A further possibility is that most of the yersinia specific ICs are phagocytosed by synovial fluid monocytes and granulocytes and are therefore not detectable in the supernatant.

The specificity of our findings is indicated by the absence of yersinia specific ICs in the synovial fluid of control patients. Nine of the controls were reactive arthritides triggered by factors other than yersiniae. Specificity of the assay has also been confirmed by inhibition experiments using *Y enterocolitica* O:3 antigens[3] and by dissociation of antigen-antibody complexes.[13] Analysis by high pressure liquid chromatography of yersinia specific ICs in the sera of patients with yersiniosis has shown that they are relatively small in size.[13]

Interestingly, two of the three patients with detectable yersinia specific ICs in the synovial fluid during the early stage of the disease continued to have joint symptoms for at least a year after the initial infection. This may indicate increased antigen load in these patients, leading to formation of yersinia specific ICs in such amounts that they become detectable by the present method. Also, in

our earlier study prolonged joint symptoms were observed in five out of the six patients with yersinia triggered reactive arthritis, who still had circulating yersinia specific ICs over eight months after the onset of infection.[3] These findings speak for the role of persisting microbial antigens in the pathogenesis of reactive arthritis.

Baldassare *et al* have studied synovial biopsy specimens from patients with Reiter's syndrome and found deposition of IgM and C3, located predominantly in the vessel wall, in 11 out of 12 samples.[14] Others have also shown the presence of immunoglobulin and complement components in the synovium of patients with Reiter's syndrome.[15 16] Therefore, taking into account the higher concentrations of circulating yersinia-IgM ICs in patients with arthritis than in those without,[3] it is possible that ICs in the synovial fluid are derived from the serum.

Another possible source of ICs in the joint is the presence of yersiniae or parts of them in the synovium, stimulating intra-articular formation of yersinia specific ICs, as is thought to occur in Lyme disease,[17] where spirochaetes were found in the synovium of two out of 17 patients[18] and in the synovial fluid of a patient with longstanding arthritis.[19] We have also recently demonstrated yersinia antigens in the synovium of a patient with yersinia triggered reactive arthritis.[20] Likewise, chlamydial antigens have been found in the synovium of patients with chlamydia triggered reactive arthritis.[21 22]

All these findings call into question definitions of postinfectious and reactive arthritis. Dumonde classifies arthritis as postinfectious when microbial antigen can be shown in the synovium or synovial fluid, and reactive in cases in which the microbial antigen is absent from the joint.[23] An example of the former is arthritis after meningococcal meningitis, where meningococcal antigens have been demonstrated in the synovium or synovial fluid white cells.[24] In this sense, it may prove that all reactive arthritides are postinfectious. Such a possibility is supported by all the indirect evidence indicating prolonged persistence of the yersinia antigens in patients with yersinia triggered reactive arthritis.[1-3 25-29]

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